Diastereoselective Synthesis of β-Branched α-Amino Acids via Biocatalytic Dynamic Kinetic Resolution

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ABSTRACT: β-branched noncanonical amino acids are valuable molecules in modern drug development efforts. However, they are still challenging to prepare due to the need to set multiple stereocenters in a stereoselective fashion and contemporary methods to achieve this often relies on the use of rare transition metal catalysis with designer ligand. Here, we report a biocatalytic transamination method to prepare a broad range of β-branched α-amino acids that proceeds with high diastereo- and enantioselectivity. Mechanistic studies show that the transformation proceeds through a dynamic kinetic resolution process that is unique to the optimal enzyme. To highlight its utility and practicality, the biocatalytic reaction is applied to the synthesis of several sp^3-rich cyclic fragments and in the first total synthesis of jomthonic acid A.

Amino acids represent one of the most indispensable and versatile building blocks in modern drug discovery.[1] In addition to its α-amino and carboxylate groups, each amino acid also contains a signature side chain that provides unique three-dimensionality and an additional structural motif for modular diversification of peptides in combinatorial synthesis. To complement the 20 canonical amino acids used in protein biosynthesis, nature also employs a variety of tailoring reactions such as hydroxylation, halogenation and methylation to further diversify these building blocks.[2] Such modification serves to modulate the physicochemical properties of the resulting noncanonical amino acid, as well as the final oligopeptide which incorporates such motif (Figure 1A). Of particular note are noncanonical amino acids (ncAAs) that contain an additional stereogenic center at the β-position due to the synergistic effects of the two adjacent stereocenters to confer additional structural rigidity. For example, the presence of a β-methylphenylalanine (β-MePhe) motif in bottromycin A2 (**1a**) was found to be vital in conferring inhibitory activity towards the prokaryotic 30S ribosomal subunit as the desmethyl analogue of the natural product (**1b**) was found to be a poor antibiotic.[3] An analogue of endomorphin bearing additional methylation at the β position of its Phe units (**3a**) was also shown to exhibit significantly improved potency and selectivity for the δ opioid receptor relative to the μ opioid receptor.[4a]

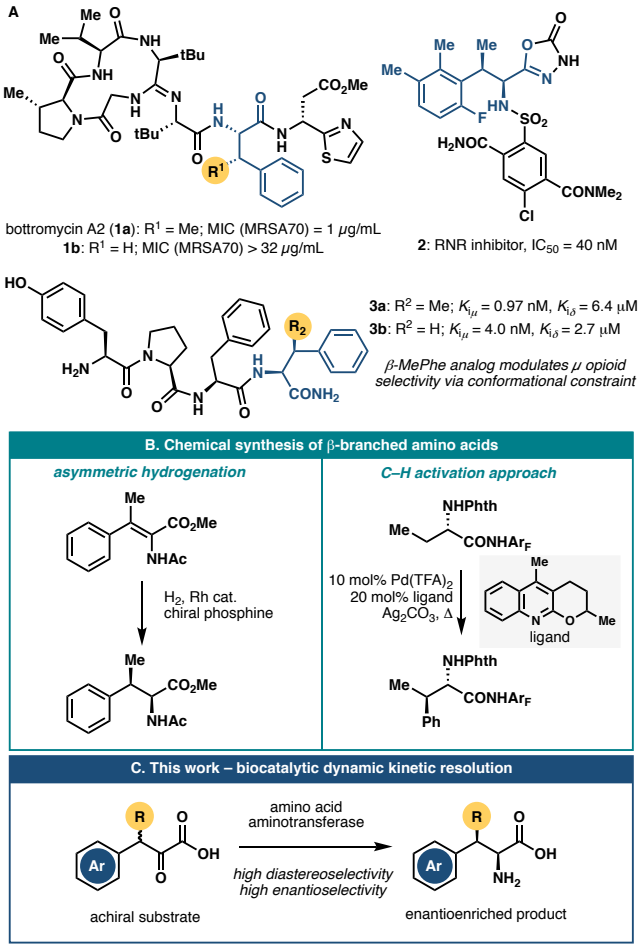


Figure 1. A. Select examples of bioactive molecules containing β-branched aromatic amino acids. **B.** Contemporary strategies to prepare β-branched aromatic amino acids via transition

metal catalysis. **C.** Proposed biocatalytic synthesis of β-branched aromatic amino acids via diastereoselective transamination of α-ketoacids.

Despite their utility in modern peptide drug discovery, β-branched ncAAs remain highly challenging to synthesize due to the need to construct multiple stereocenters. Chemical synthesis of ncAAs emphasizes on the use of asymmetric transformations to set the α-stereocenter (Figure 1B) and has resulted in the development of several practical strategies, including asymmetric hydrogenation,[5] asymmetric Strecker reaction,[6] and the use of chiral auxiliaries in either polar- or radical-based reactions.[7] However, synthesis of β-branched ncAAs using any of these strategies will require a separate step to establish the stereocenter at the β position. In the case of radical-based method, facile racemization at the radical center leads to a 1:1 diastereomeric mixture at the β position. Recent advances in C–H functionalization have enabled the synthesis of β-branched ncAAs through direct arylation or alkylation of the β-carbon.[8] However, such approaches often require the use of rare transition metal catalysts at high catalyst loading and pre-installation of a directing group and at times, suffer from sub-optimal diastereoselectivity.

Enzymatic transformations, by virtue of their unparalleled selectivity profile, represent an attractive solution to the challenges associated with ncAA synthesis.[9] Indeed, a gamut of biocatalytic processes has been developed in recent years in this area. Despite these advances, biocatalytic processes that are able to generate multiple stereocenters in a single transformation remain rare. Arnold and co-workers recently reported the use of engineered tryptophan synthases for the formation of β-alkyl tryptophan analogues.[10] However, this approach is limited to indole-containing β-branched ncAAs. Seebeck and co-workers[11] also described the use of a self-contained enzymatic cascade for asymmetric β-methylation of α-amino acids, though this method requires the use of three enzymes in the cascade at high enzyme loading (2 mol% loading) and proceeds with low total turnover numbers overall. Here, we report a biocatalytic dynamic kinetic resolution (DKR) approach for the synthesis of β-branched aromatic amino acids that (1) establishes two contiguous stereocenters with complete diastereocontrol, (2) proceeds with excellent enantioselectivity towards the L-amino acid product and high catalyst efficiency, and (3) employs readily available α-ketoacid substrates (Figure 1C). Key in the reaction design is the identification of a suitable thermophilic enzyme that is able to withstand the non-physiological conditions required while also exhibiting several unique features to enable the realization of a DKR process.

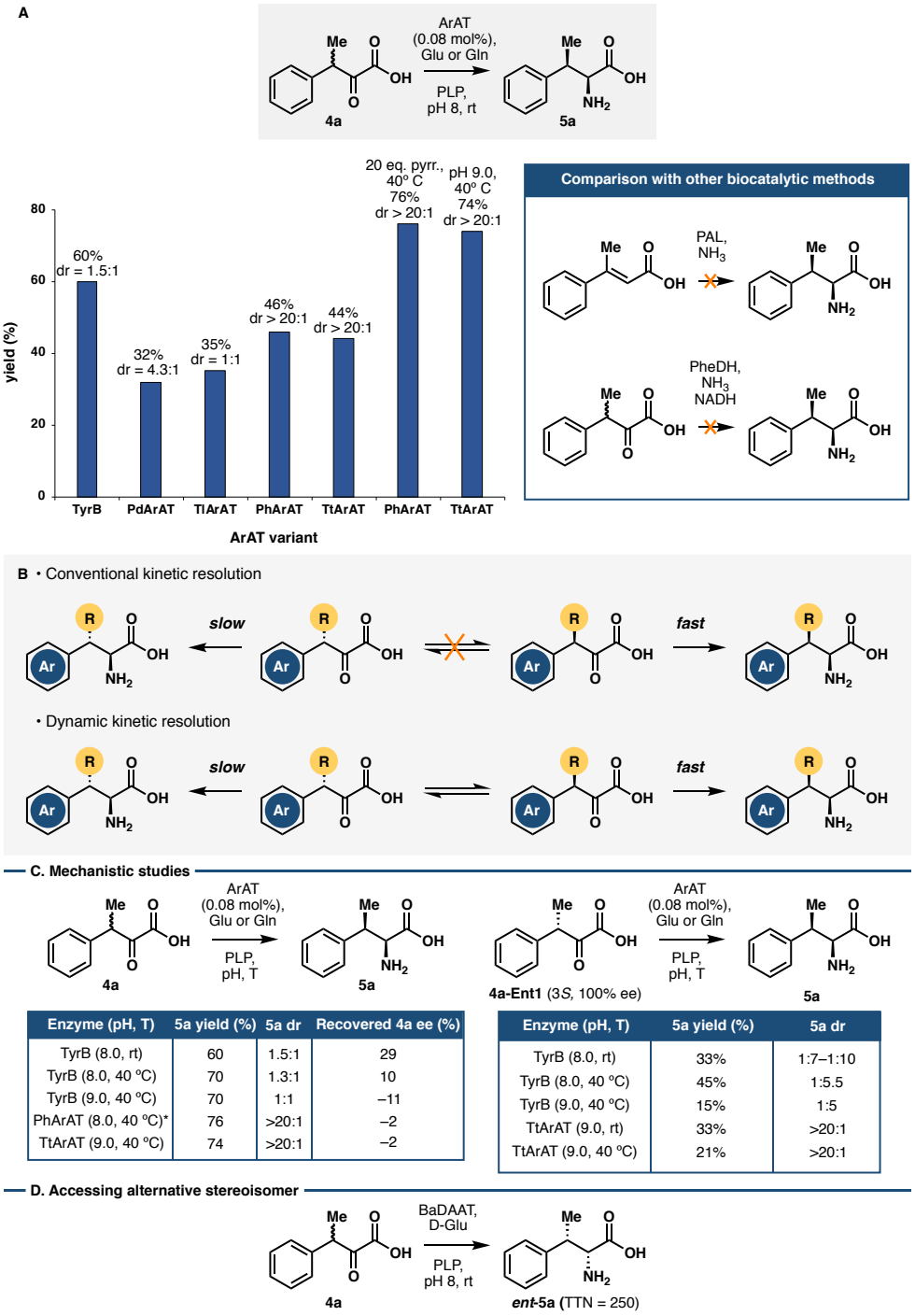
Aromatic amino acid aminotransferases (ArATs) are pyridoxal-phosphate (PLP)-dependent enzymes that are responsible for the biosynthesis of phenylalanine via transamination of phenylpyruvate with other amino acids as the amine donor. Several lines of evidence from the biosynthetic literature hint at the promiscuity of these aminotransferases. In their investigation on the biosynthetic origins of the β-MePhe moiety of mannopeptimycin,[12] Li and co-workers were able to identify a dedicated methyltransferase that methylates phenylpyruvate at the β position but were not able to find an aminotransferase within the biosynthetic gene cluster. Hypothesizing that an enzyme from primary metabolism is responsible for the latter transformation, the authors showed that TyrB, an ArAT from *E. coli*, is capable of converting β-methylphenylpyruvic acid to β-MePhe, albeit as a diastereomeric mixture at the β position. A similar observation was also made by Piel and co-workers in their biosynthetic studies on hormaomycin.[13] Importantly, these observations led us to hypothesize that ArATs are capable of accepting related pyruvate substrates that contain additional substituents at the β position to produce β-branched aromatic amino acids and that we would be able to identify a suitable ArAT that could catalyze this process with high diastereoselectivity.

Due to the importance of aromatic amino acids, ArAT is present in all domains of life and TyrB homologs have been identified from various species. Nevertheless, these ArATs have enjoyed only limited biocatalytic application. We began our investigation by examining the synthetic utility of a panel of TyrB homologs in the conversion of phenylpyruvate to β-MePhe. Of special interest are ArATs belonging to thermophilic bacteria due to the well-known benefits associated with thermostable enzymes in biocatalysis, namely the ability to withstand harsh reaction conditions, as well as superior evolvability[14] for future engineering efforts. With this feature in mind, three thermophilic enzymes, TlArAT (from *T. litoralis*),[15a] PhArAT (from *P. horikoshii*)[15b] and TtArAT (from *T. thermophilus*),[15c] were included in our initial screening. In addition to their thermophilicity, these three enzymes have also been structurally characterized, though their use in biotechnology has not been explored before.

Our initial screening with β-methylphenylpyruvic acid (**4a**, Figure 2A) revealed that while transamination with TyrB was able to deliver the desired product in 60% yield (total turnover number, TTN = 750), it proceeded with poor diastereoselectivity (dr = 1.5:1). A similar observation was obtained in reactions with PdArAT (from *P. denitrificans*)[15d] and TlArAT, whereby product **5a** was obtained only in 32% and 35% yields and moderate to poor diastereoselectivity. In contrast, reactions with PhArAT and TtArAT provided excellent diastereoselectivity for the desired product, though only moderate yields were observed. We also tested phenylalanine ammonia lyase (PAL) from *A. variabilis*[16] and phenylalanine dehydrogenases (PheDHs)[17] from *B. sphaericus* and *C. thermarum* for the preparation of **5a**, but all reactions failed to provide the desired product. At this point, two pathways could potentially be operative in the ArAT-catalyzed transamination (Figure 2B). First, the reaction could proceed under a conventional kinetic resolution (KR) whereby the two enantiomers of **4a** are not interconvertible under the reaction conditions and that any diastereoselectivity observed is governed by the intrinsic preference of the active site of a given enzyme. Alternatively, the two enantiomers could be rapidly interconverting to establish a DKR process.

To determine which of these pathways is operative, unreacted **4a** was isolated at the end of the reactions with TyrB, PhArAT and TtArAT and submitted to enantiomeric excess (ee) measurements using chiral supercritical fluid chromatography (SFC). Intriguingly, unreacted **4a** from the TyrB-catalyzed transamination displays 29% ee, which stood in stark contrast to the completely racemic material obtained when unreacted **4a** from

reactions with PhArAT and TtArAT was tested (Figure 2C). This observation suggests that a DKR process might be operative with PhArAT and TtArAT while transamination with TyrB might proceed through a conventional KR process. Gratifyingly, further optimization of transamination with PhArAT and TtArAT improved the isolated yield of **5a** to more than 70% without any loss in diastereoselectivity. The use of 20 equivalent of pyrrolidine at 40 °C proved beneficial in reaction with PhArAT, while TtArAT provided optimal conversion at 40 °C and pH 9.0. Preliminary investigations suggest that TtArAT is more promiscuous than PhArAT and the former was chosen for subsequent investigation.



Enzyme (pH, T)	5a yield (%)	5a dr	Recovered 4a ee (%)
TyrB (8.0, rt)	60	1.5:1	29
TyrB (8.0, 40 °C)	70	1.3:1	10
TyrB (9.0, 40 °C)	70	1:1	–11
PhArAT (8.0, 40 °C)*	76	>20:1	–2
TtArAT (9.0, 40 °C)	74	>20:1	–2

Enzyme (pH, T)	5a yield (%)	5a dr
TyrB (8.0, rt)	33%	1:7–1:10
TyrB (8.0, 40 °C)	45%	1:5.5
TyrB (9.0, 40 °C)	15%	1:5
TtArAT (9.0, rt)	33%	>20:1
TtArAT (9.0, 40 °C)	21%	>20:1

Figure 2. A. Screening of various ArATs for the transamination of **4a**. TyrB: L-ArAT from *E. coli*, PdArAT: L-ArAT from *P. denitrificans*, TlArAT: L-ArAT from *T. litoralis*, PhArAT: L-ArAT from *P. horikoshii*, TtArAT: L-ArAT from *T. thermophilus*, pyrr.: pyrrolidine. **B.** Two potential resolution scenarios for the conversion of **4a** to **5a**. **C.** Verification of the different types of kinetic resolution process that are operative with TyrB and TtArAT through mechanistic studies. **D.** Synthesis of ***ent*-5a** via biocatalytic transamination of **4a** with BaDAAT (D-ArAT from *Bacillus* sp. Strain YM-1). Standard conditions for transamination: α-ketoacid (10 mM, 1 equiv), amine donor (30 mM, 3 equiv), PLP (0.25 mM, 2.5 mol%), ArAT (0.008 mM, 0.08 mol%), buffer (50 mM pH 8.0 KPi or 50 mM Tris pH 9.0, 3 mL total volume), 24 h. *20 equiv of pyrrolidine added to the reaction.

Further studies were performed to ascertain the type of kinetic resolution that is operative with TyrB and TtArAT. First, pure 3*S* and 3*R* enantiomers of **4a** (**4a-Ent1** and **4a-Ent2** respectively) were obtained via preparative chiral SFC separation and submitted to reactions with TyrB and TtArAT. Transamination of **4a-Ent1** with TyrB led to only minor formation of the *syn* diastereomer **5a**, with the *anti* diastereomer formed as the major product. Additionally, the reaction became less diastereoselective at elevated temperature or pH. In contrast, TtArAT-catalyzed transamination of **4a-Ent1** proceeded with stereoinversion at C3, providing the *syn* diastereomer **5a** exclusively. As expected, all reactions with **4a-Ent2** formed *syn* **5a** as the major diastereomer regardless of the enzyme used, though it is worth noting that reaction yields and conversions of all enzymes with **4a-Ent2** were considerably higher than those with **4a-Ent1**. Control experiments in the absence of enzyme showed that enantiopure **4a** undergoes slow racemization at pH 8 and that this process becomes more prominent at increased pH or temperature. This observation led us to revise our proposal for the different kinetic resolutions with TyrB and TtArAT.

Contrary to our initial hypothesis, the two substrate enantiomers are able to interconvert–albeit slowly–under standard reaction conditions with TyrB. However, TyrB shows only limited ability to discriminate between the enantiomers in its active site. While racemization of substrate enantiomers takes place more rapidly under optimal conditions with TtArAT, productive catalysis with the enzyme only takes place with the 3*R* enantiomer. Though this might seem to indicate a high level of stereodiscrimination in its active site, we still cannot rule out the possibility that TtArAT is actually able to accept both substrate enantiomers in its active site but contains additional features that allow rapid convergence into one enantiomer following the formation of enzyme-substrate complex. Further mechanistic and structural studies to elucidate the finer details of this process are ongoing. Finally, the differential rates of racemization at different pH and temperature might explain the change in diastereoselectivity in reaction with TyrB under different conditions and the differential rates of conversion of the two enantiomers of **4a** under optimized conditions for all enzymes. Preliminary screening of D-ArATs also revealed that an enzyme from *Bacillus* sp. strain YM-1 (BaDAAT)[18] is capable of catalyzing the production of **ent-5a** from **4a** in moderate TTN, suggesting the possibility of accessing all other possible diastereomers of **5a** through further genome mining and/or enzyme engineering.

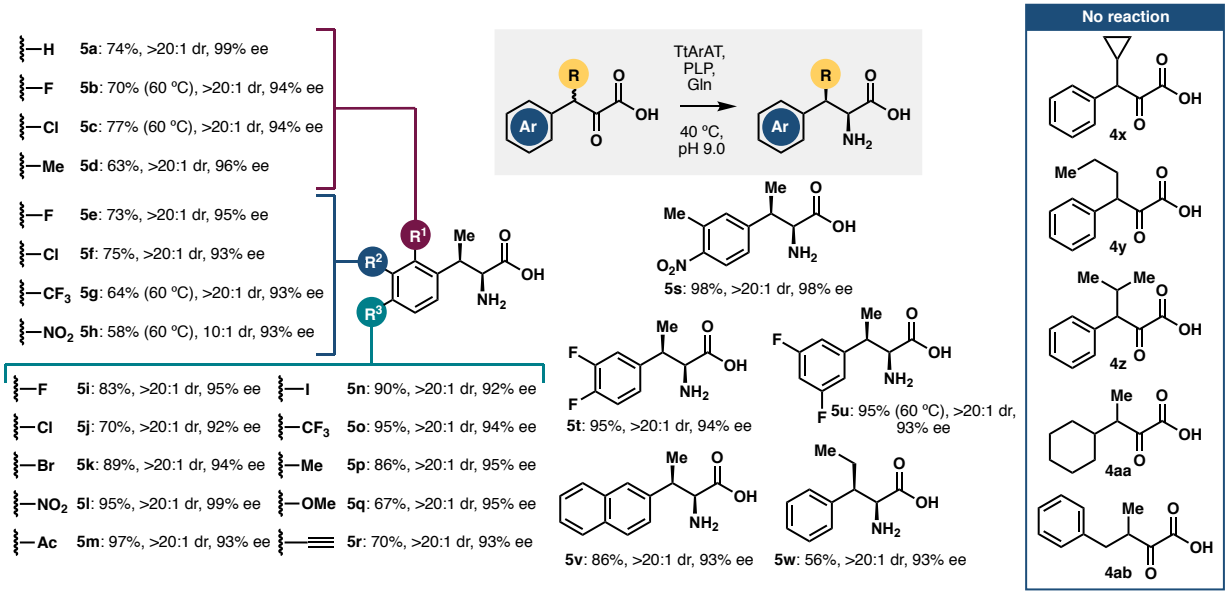


Figure 3. Substrate scope of biocatalytic transamination with TtArAT. Reaction conditions: α-ketoacid (10 mM, 1 equiv), Gln (30 mM, 3 equiv), PLP (0.25 mM, 2.5 mol%), TtArAT (0.008 mM, 0.08 mol%), Tris buffer (pH 9.0, 50 mM, 15 mL total volume), 24 h at 40 or 60 °C. Yields refer to isolated yields after C18 purification. See Supporting Information for details on dr and ee measurements.

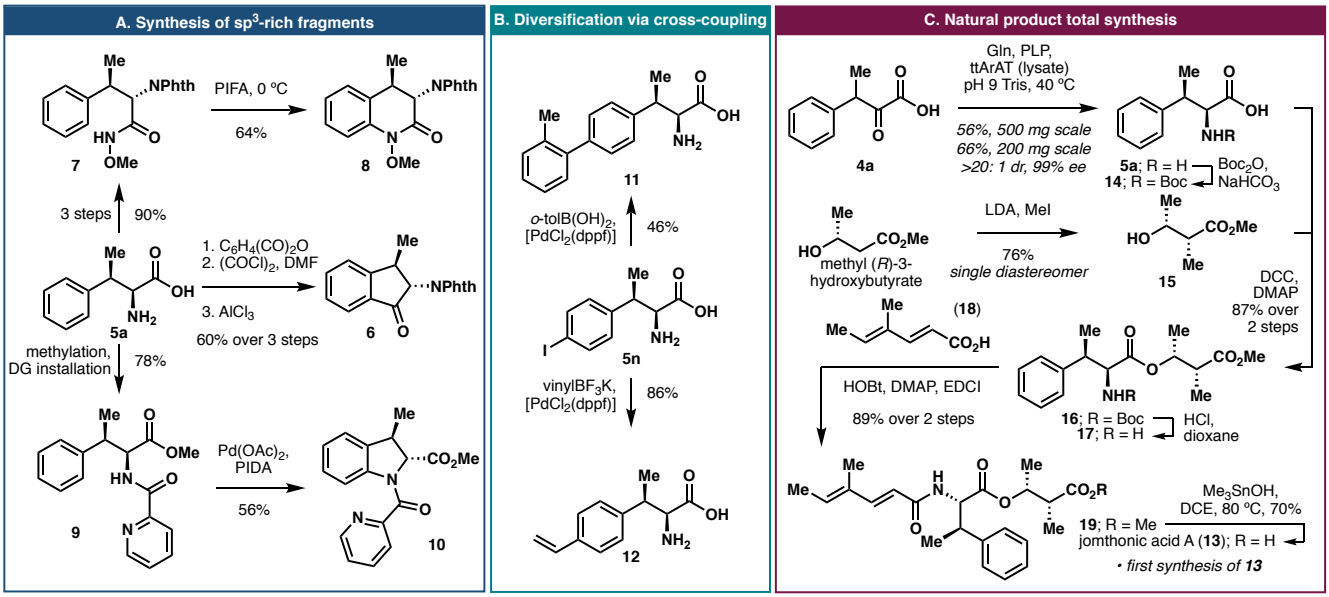
Following optimization, the scope and limitations of the transformation were tested on various substrates (Figure 3). Productive reactions were observed with a variety of substrates bearing additional functional groups on their aromatic ring. In general, substitution at the *para* position on the ring (relative to the amino acid alkyl chain) is more tolerated than that at the *ortho* and *meta* positions. In several cases, the use of elevated reaction temperature (60 °C) was found advantageous in improving the reaction yields, demonstrating the benefit of employing a thermophilic enzyme in the reaction. Comparison of reaction yields obtained for products **5k**–**m**, **5o**, and **5q** suggests slight preference for substrates bearing electron withdrawing groups. However, no strong correlation between the Hammett parameters of the respective ring substituents and yields could be observed. Thus, any variation in activity likely arises primarily due to differences in steric interactions within the active site. Interestingly, increasing the size of the aryl ring to a naphthyl group led to only a small decrease in reaction yield.

The enzymatic transformation is also well-suited for the production of β-MePhe analogues with multiple substituents (e.g. **5s**–**u**) in high yields. This feature is expected to be useful for

further derivatization and manipulation of the aryl ring to arrive at more complex structures. While a small change from methyl to ethyl at the β-position is tolerated, more drastic deviations such as the introduction of a propyl, cyclopropyl and isopropyl at this position led to no reaction. This observation suggests that the active site of TtArAT is highly sensitive to steric effects at the β position of the substrate. At present, the transamination reaction is limited to the production of β-branched Phe analogues as substrates containing aliphatic chains or heteroaromatics did not participate in the reaction. Nevertheless, excellent diastereoselectivity and enantioselectivity were observed in all productive reactions. Another attractive feature of this method is the ability to attain high conversion and yield with a single enzyme system. In contrast, prior approaches to prepare ncAA through biocatalytic reductive amination or transamination often require the use of additional enzymes for cofactor recycling, amine donor recycling or byproduct removal to drive the reaction equilibrium forward.[9]

We next sought to showcase the synthetic utility and versatility of this biocatalytic platform in the production of various sp^3-rich cyclic fragments (Scheme 1A). Such complex structures are rich in three-dimensionality and are becoming increasingly valuable building blocks to "escape from flatland" in combinatorial synthesis and drug discovery.[19] Following appropriate protecting group introduction, a derivative of **5a** readily underwent a Friedel-Crafts cyclization[20] to generate an indanone product containing two stereocenters (**6**). Introduction of N-methoxyamide auxiliary on **5a** facilitated an oxidative cyclization in the presence of [bis(trifluoroacetoxy)iodo]benzene (PIFA)[21] to afford a multiply substituted 3,4-dihydroisoquinoline product (**8**). In a similar fashion, a chiral indoline containing two defined stereocenters (**10**) could be synthesized through the use of palladium-catalyzed C–H amination approach developed by Chen.[22] The ability to produce halogen-containing β-MePhe derivatives using this method also facilitated the synthesis of more complex products through metal-catalyzed cross-coupling (Scheme 1B).[23] For example, the use of Suzuki coupling on unprotected **5n** readily afforded biaryl product **11** or styrenyl product **12**.

Scheme 1. A. Derivatization of β-MePhe (**5a**) for the synthesis of several sp^3-rich fragments. **B.** Diversification of product **5n** through Pd-catalyzed Suzuki coupling. **C.** Application of biocatalytic transamination with TtArAT in the total synthesis of jomthonic acid A (**13**).



Finally, we demonstrated the viability and practicality of this method for large-scale production of β-MePhe (**5a**) to meet the material supply demands of a total synthesis campaign (Scheme 1C). Here, jomthonic acid A (**13**), a soil-derived natural product with antidiabetic and antiatherogenic activities,[24] was chosen as synthetic target. Our approach commenced with the use of TtArAT-catalyzed transamination to produce **5a** on more than 500 mg scale in 56–66% yield. For subsequent synthetic manipulations, **5a** was submitted to a routine Boc protection. In parallel, alcohol **15** was prepared via a diastereoselective α-methylation of methyl (*R*)-3-hydroxybutyrate.[25] Coupling of **14** and **15** in the presence of DCC and DMAP proceeded uneventfully to afford ester **16**, which was treated with HCl in dioxane to unmask its free amine. Following peptide coupling of **17** with acid **18**, selective methyl ester hydrolysis was achieved through the use of Me_3SnOH to complete the first synthesis of jomthonic acid A.

In conclusion, by leveraging the intrinsic sequence diversity of ArATs, we identified a suitable thermophilic ArAT for the biocatalytic production of β-branched aromatic amino acids, which establishes two adjacent stereocenters in a single transformation through a unique DKR process. The transformation is highly efficient and practical, enabling further diversification of the products obtained to generate sp^3-rich fragments for potential applications in drug discovery, as well as incorporation of the process in a chemoenzymatic synthesis. Though our substrate scope examination identified several problematic substrate classes, we envision that this issue can be addressed through further genome mining and enzyme engineering efforts. For example, the biocatalytic synthesis of all-aliphatic β-

branched amino acids can potentially be achieved through the use of a similar DKR strategy with IlvE, a family of branched-chain-amino-acid aminotransferases which are responsible for the biosynthesis of leucine, isoleucine and valine.[11,26] Additionally, our preliminary results with BaDAAT suggest that access to alternative product stereoisomers will be possible through exploration of other types of aminotransferases. Further studies in these areas towards the biocatalytic synthesis of more complex branched amino acids are actively being pursued in our laboratory.

ASSOCIATED CONTENT

Supporting Information

Experimental details, analytical data, ^{1}H and ^{13}C NMR data (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally.

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Notes

F.L., L.-C.Y. and H.R. have applied for a provisional patent for this work.

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REFERENCES

1. Henninot, A.; Collins, J.C.; Nuss, J. M. The Current State of Peptide Drug Discovery: Back to the Future? *J. Med. Chem.* **2018**, *61*, 1382–1414.

2. Hedges, J. B.; Ryan, K. S. Biosynthetic Pathways to Nonproteinogenic α-Amino Acids. *Chem. Rev.* **2020**, *120*, 3161–3209.

3. Yamada, T.; Yagita, M.; Kobayashi, Y.; Sennari, G.; Shimamura, H.; Matsui, H.; Horimatsu, Y.; Hanaki, H.; Hirose, T.; Omura, S.; Sunazuka, T. Synthesis and Evaluation of Antibacterial Activity of Bottromycins. *J. Org. Chem.* **2018**, *83*, 7135–7149.

4. (a) Tömböly, C.; Köver, K. E.; Péter, A.; Tourwé, D.; Biyashev, D.; Benyhe, S.; Borsodi, A.; Al-Khrasani, M.; Rónai, A. Z.; Tóth, G. Structure–Activity Study on the Phe Side Chain Arrangement of Endomorphins Using Conformationally Constrained Analogues. *J. Med. Chem.* **2004**, *47*, 735–743. (b) Miyahara, S.; Hiroyuki, U.; Shoki, H.; Yoshio, O. Sulfonamide Compound or Salt Thereof. WIPO Patent Application WO/2017/209155, December 7, 2017.

5. (a) Burk, M. J.; Gross, M. F.; Martinez, J. P. Asymmetric Catalytic Synthesis of β-Branched Amino Acids via Highly Enantioselective Hydrogenation of α-Enamides. *J. Am. Chem. Soc.* **1995**, *117*, 9375–9376. (b) Ji, J.; Chen, C.; Cai, J.; Wang, X.; Zhang, K.; Shi, L.; Lv, H.; Zhang, X. Highly Enantioselective Synthesis of Non-Natural Aliphatic α-Amino Acids via Asymmetric Hydrogenation. *Org. Biomol. Chem.* **2015**, *13*, 7624–7627. (c) Roff, G. J.; Lloyd, R.-C.; Turner, N. J. A Versatile Chemo-Enzymatic Route to Enantiomerically Pure β-Branched α-Amino Acids. *J. Am. Chem. Soc.* **2004**, *126*, 4098–4099.

6. Zuend, S. J.; Coughlin, M. P.; Lalonde, M. P.; Jacobsen, E. N. Scalable Catalytic Asymmetric Strecker Syntheses of Unnatural α-Amino Acids. *Nature* **2009**, *461*, 968–970.

7. (a) Davis, F. A.; McCoull, W. Concise Asymmetric Synthesis of a-Amino Acid Derivatives from N-Sulfinylimino Esters. *J. Org. Chem.* **1999**, *64*, 3396–3397. (b) Beenen, M. A.; Weix, D. J.; Ellman, J. A. Asymmetric Synthesis of Protected Arylglycines by Rhodium-Catalyzed Addition of Arylboronic Acids to N-tert-Butanesulfinyl Imino Esters. *J. Am. Chem. Soc.* **2006**, *128*, 6304–6305. (c) Wangweerawong, A.; Hummel, J. R.; Bergman, R. G.; Ellman, J. A. Preparation of Enantiomerically Pure Perfluorobutanesulfinamide and Its Application to the Asymmetric Synthesis of α-Amino Acids. *J. Org. Chem.* **2016**, *81*, 1547–1557. (d) Ni, S.; Garrido-Castro, A. F.; Merchant, R. R.; deGruyter, J. N.; Schmitt, D. C.; Mousseau, J. J.; Gallego, G. M.; Yang, S.; Collins, M. R.; Qiao, J. X.; Yeung, K.-S.; Langley, D. R.; Poss, M. A.; Scola, P. M.; Qin, T.; Baran, P. S. A General Amino Acid Synthesis Enabled by Innate Radical Cross-Coupling. *Angew. Chem. Int. Ed.* **2018**, *57*, 14560–14565.

8. (a) Chen. G.; Shigenari, T.; Jain, P.; Zhang, Z.; Jin, Z.; He, J.; Li, S.; Mapelli, C.; Miller, M. M.; Poss, M. A.; Scola, P. M.; Yeung, K.-S.; Yu, J.-Q. Ligand-Enabled β-C–H Arylation of α-Amino Acids Using a Simple and Practical Auxiliary. *J. Am. Chem. Soc.* **2015**, *137*, 3338–3351. (b) Zhang. S.-Y.; Li, Q.; He, G.; Nack, W. A.; Chen, G. Stereoselective Synthesis of β-Alkylated α-Amino Acids via Palladium-Catalyzed Alkylation of Unactivated Methylene C(sp^3)–H Bonds with Primary Alkyl Halides. *J. Am. Chem. Soc.* **2013**, *135*, 12135–12141.

9. Xue, Y.-P.; Cao, C.-H.; Zheng, Y.-G. Enzymatic Asymmetric Synthesis of Chiral Amino Acids. *Chem. Soc. Rev.* **2018**, *47*, 1516–1561.

10. Boville, C. E.; Scheele, R. A.; Koch, P.; Brinkmann-Chen, S.; Buller, A. R.; Arnold, F. H. Engineered Biosynthesis of β-Alkyl Tryptophan Analogs. *Angew. Chem. Int. Ed.* **2018**, *57*, 14764–14768.

11. Liao, C.; Seebeck, F. P. Asymmetric β-Methylation of L- and D-α-Amino Acids by a Self-Contained Enzyme Cascade. *Angew. Chem. Int. Ed.* **2020**, *59*, 7184–7187.

12. Huang, Y,-T.; Lyu, S.-Y.; Chuang, P.-H.; Hsu, N.-S.; Li, Y.-S.; Chan, H.-C.; Huang, C.-J.; Liu, Y.-C.; Wu, C.-J.; Yang, W.-B.; Li, T.-L. In Vitro Characterization of Enzymes Involved in the Synthesis of Nonproteinogenic Residue (2*S*,3*S*)-β-Methylphenylalanine in Glycopeptide Antibiotic Mannopeptimycin. *ChemBioChem* **2009**, *10*, 2480–2487.

13. Hofer, I.; Crusemann, M.; Radzom, M.; Geers, B.; Flachshaar, D.; Cai, X.; Zeeck, A.; Piel, J. Insights into the Biosynthesis of Hormaomycin, an Exceptionally Complex Bacterial Signaling Metabolite. *Chem. Biol.* **2011**, *18*, 381–391.

14. Bloom, J. D.; Labthavikul, S. T.; Arnold, F. H. Protein Stability Promotes Evolvability. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5869–5874.

15. (a) Andreotti, G.; Cubellis, M. V.; Nitti, G.; Sannia, G.; Mai, X.; Marino, G.; Adams, M. W. W. Characterization of Aromatic Aminotransferases from the Hyperthermophilic Archaeon *Thermococcus litoralis*. *Eur. J. Biochem*. **1994**, *220*, 543–549. (b) Matsui, I.; Matsui, E.; Sakai, Y.; Kikuchi, H.; Kawabayasi, Y.; Ura, H.; Kawaguchi, S.; Kuramitsu, S.; Harata, K. The Molecular Structure of Hyperthermostable Aromatic Aminotransferase with Novel Substrate Specificity from *Pyrococcus horikoshii*. *J. Biol. Chem*. **2000**, *275*, 4871–4879. (c) Hosono, A.; Mizuguchi, H.; Hayashi, H.; Goto, M.; Miyahara, I.; Hirotsu, K.; Kagamiyama, H. Glutamine:Phenylpyruvate Aminotransferase from an Extremely Thermophilic Bacterium, *Thermus thermophilus* HB8. *J. Biochem.* **2003**, *134*, 843–851. (d) Okamoto, A.; Nakai, Y.; Hayashi, H.; Hirotsu, K.; Kagamiyama, H. Crystal Structures of *Paracoccus*

denitrificans Aromatic Amino Acid Aminotransferase: A Substrate Recognition Site Constructed by Rearrangement of Hydrogen Bond Network. *J. Mol. Biol.* **1998**, *280*, 443–461.
16. Lovelock, S. L.; Lloyd, R. C.; Turner, N. J. Phenylalanine Ammonia Lyase Catalyzed Synthesis of Amino Acids by an MIO-Cofactor Independent Pathway. *Angew. Chem. Int. Ed.* **2014**, *53*, 4652–4656.
17. (a) Okazaki, N.; Hibino, Y.; Asano, Y.; Ohmori, M.; Numao, N.; Kondo, K. Cloning and Nucleotide Sequencing of Phenylalanine Dehydrogenase Gene of Bacillus sphaericus. *Gene* **1988**, *63*, 337–341. (b) Pushpanath, A.; Siirola, E.; Bornadel, A.; Woodlock, D.; Schell, U. Understanding and Overcoming the Limitations of *Bacillus badius* and *Caldalkalibacillus thermarum* Amine Dehydrogenases for Biocatalytic Reductive Amination. *ACS Catal.* **2017**, *7*, 3204–3209.
18. Walton, C. J. W.; Parmeggiani, F.; Barber, J. E. B.; McCann, J. L.; Turner, N. J.; Chica, R. A. Engineered Aminotransferase for the Production of D-Phenylalanine Derivatives Using Biocatalytic Cascades. *ChemCatChem* **2018**, *10*, 470–474.
19. (a) Lovering, F.; Bikker, J.; Humblet, C. Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success. *J. Med. Chem.* **2009**, *52*, 6752–6756. (b) Devine, P. N.; Howard, R. M.; Kumar, R.; Thompson, M. P.; Truppo, M. D.; Turner, N. J. Extending the Application of Biocatalysis to Meet the Challenges of Drug Development. *Nature. Rev. Chem.* **2018**, *2*, 409–421. (c) Ramsden, J. I.; Cosgrove, S. C.; Turner, N. J. Is It Time for Biocatalysis in Fragment-Based Drug Discovery? *Chem. Sci.* **2020**, *11*, 11104–11112.
20. Amano, Y.; Inoue, K.; Nishiyama, S. Oxidative Access to Quinolinone Derivatives with Simultaneous Rearrangement of Functional Groups. *Synlett* **2008**, *1*, 134–136.
21. McClure, D. E.; Lumma, P. K.; Arison, B. H.; Jones, J. H.; Baldwin, J. J. 1,4-Oxazines via Intramolecular Ring Closure of β-Hydroxydiazoacetamides: Phenylalanine to Tetrahydroindeno[1,2-b]-1,4-oxazin-3(2H)-ones. *J. Org. Chem.* **1983**, *48*, 2675–2679.
22. He, G.; Lu, C.; Zhao, Y.; Nack, W. A.; Chen, G. Improved Protocol for Indoline Synthesis via Palladium-Catalyzed Intramolecular C(sp^2)–H Amination. *Org. Lett.* **2012**, *14*, 2944–2947.
23. (a) Roy, A. D.; Grüschow, S.; Cairns, N.; Goss, R. J. M. Gene Expression Enabling Synthetic Diversification of Natural Products: Chemogenetic Generation of Pacidamycin Analogs. *J. Am. Chem. Soc.* **2010**, *132*, 12243–12245. (b) Runguphan, W.; O'Connor, S. E. Diversification of Monoterpene Indole Alkaloid Analogs through Cross-Coupling. *Org. Lett.* **2013**, *15*, 2850-1853. (c) Durak, L. J.; Payne, J. T.; Lewis, J. C. Late-Stage Diversification of Biologically Active Molecules via Chemoenzymatic C–H Functionalization. *ACS Catal.* **2016**, *6*, 1451–1454. (d) Latham, J.; Henry, J.-M.; Sharif, H. H.; Menon, B. R. K.; Shepherd, S. A.; Greaney, M. F.; Micklefield, J. Integrated Catalysis Opens New Arylation Pathways via Regiodivergent Enzymatic C–H Activation. *Nature Commun.* **2016**, *7*, 11873.
24. Igarashi, Y.; Yu, L.; Ikeda, M.; Oikawa, T.; Kitani, S.; Nihira, T.; Bayanmunkh, B.; Panbangred, W. Jomthonic Acid A, a Modified Amino Acid from a Soil-Derived Streptomyces. *J. Nat. Prod.* **2012**, *75*, 986–990.
25. Clark, J. S.; Yang, G.; Osnowski, A. P. Synthetis of the C-1–C-17 Fragment of Amphidinolides C, C2, C3, and F. *Org. Lett.* **2013**, *15*, 1460–1463.
26. Yu, X.; Wang, X.; Engel, P. C. The Specificity and Kinetic Mechanism of Branched-Chain Amino Acid Aminotransferase from *Escherichia coli* Studied with a New Improved Coupled Assay Procedure and the Enzyme's Potential for Biocatalysis. *FEBS J.* **2014**, *281*, 391–400.
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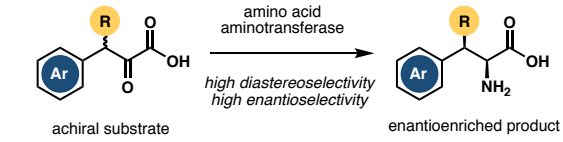
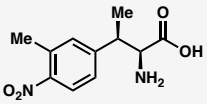
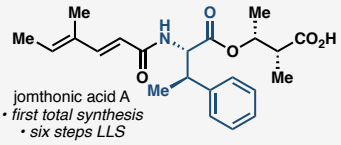


achiral substrate

enantioenriched product



98%, >20:1 dr, 98% ee



jomthonic acid A

- *first total synthesis*
- *six steps LLS*